U.S. Department of Agriculture
Forest Service Research Paper SO-110

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Forest Service
U. S. Department of Agriculture

1975

XYLEM DEVELOPMENT OF LOBLOLLY PINE DURING IRRIGATION AND SIMULATED DROUGHT

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In 27-year-old loblolly pines growing in southeastern Arkansas, soil-water stress reduced the total number of cells produced and appeared to reduce the width of latewood cells. At breast height, transition from earlywood to latewood occurred in early June and was little affected by irrigation but may have been influenced by root injury from trenching. In trees subjected to drought, flat latewood cells first appeared in July; subsequent irrigation stimulated the production of wider latewood cells but not until 3 weeks had passed. In general, the transition from earlywood to latewood was marked by a sharp increase in the time that cells remained in the maturing xylem zone. Trends in wood formation at the base of the live crown were similar to those at breast height but less well defined.

Additional keywords: *Pinus taeda,* soil-water stress, tracheid, wood formation.

Soil moisture status influences formation and development of xylem elements in conifers (Kramer 1964, Zahner 1963, 1968). Larson (1964) suggested that internal water stress directly inhibits production of growth regulators in the crown and thereby modifies cambial activity. Whitmore and Zahner (1966) showed that water stress influences anatomical features of the annual ring of red pine (*Pinus resinosa* Ait.). In work with Scotch pine, *P. sylvestris* L., they (1967) further demonstrated that internal moisture deficit affected tracheid wall metabolism of cambial derivatives independently of regulators produced in the crown.

This paper reports effects of soil-water stress on amount, periodicity, and duration of development of tracheids at breast height (1.4 m aboveground) and at the base of the crown in pulpwood-size loblolly pines (*P. taeda* L.).

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METHODS

A 27-year-old, natural stand near Crossett, Arkansas, was selected for study. The site index is 27.4 m at age 50 years. Basal area averaged 21.8 square meters per hectare. Trees ranged from 25.4 to 30.5 cm in diameter and from 18.0 to 20.4 m in height. Live crown lengths averaged 45 percent of total height. A dense hardwood understory covered the area.

The soil is Grenada silt loam (Glossic Fragiudalf), an imperfectly drained loess underlain by a weak fragipan at a depth of 46 to 61 cm. Available water-holding capacity (0.06 to 15 atm of soil water tension) is estimated to be 30 cm of water in the 0- to 122-cm soil layer.

In April 1965, 15 trees were grouped into five clusters or plots of three trees apiece, and one of five treatments was randomly assigned to each plot:

Drought (D). Continuous surface drought, May through December. Plot trenched and sheltered from rain by polyethylene cover.

Irrigation (I). Surface 30 cm of soil maintained near field capacity by supplemental irrigation, May through December. Plot trenched and irrigated weekly by rotary sprinklers.

Drought + Irrigation (DI). Drought imposed from May 1 to July 15 by polyethylene cover; irrigation from July 16 through December. Plot trenched.

Trenched Control (TC). Natural rainfall; plot trenched.

Untrenched Control (UC). Natural rainfall; plot not trenched.

Trenches were 46 cm wide by 122 cm deep, and at least 3 m from the boles of study trees. During imposed droughts the polyethylene sheets shunted rainwater into the trench; a catchment basin was dug in the lowest part of the trench to take the runoff.

Water content in the 0- to 122-cm soil layer was measured weekly with a neutron probe. Rain was recorded daily at an adjacent weather station. Irrigation water was measured with rain gages.

Diameter growth at breast height and at the base of the crown of each tree was measured weekly with aluminum-band dendrometers to the nearest 0.2 mm. To study tracheid development at both bole positions, wood samples (1 cm wide x 2 cm long x 1 cm thick) extending inward 2 to 3 growth rings were extracted with a chisel at 1- to 4-week intervals from May 10, 1965, to January 24, 1966. The samples were immediately fixed in formalin-acetic acid-alcohol, then infiltrated with celloidin (Sass 1958). To detect lignification, transverse sections 20 to 30μ thick were cut and subjected to a phloroglucin-hydrochloric acid solution, which turns lignified cell walls red-violet. Transverse sections 15 to 25μ thick were cut and stained with safranin and fast green to help identify developing zones in the newly differentiating xylem. Although many samples were extracted from a small area on the boles of all trees, no abnormalities in wood formation were apparent.

Three zones of differentiating xylem (Wilson et al. 1966) were studied: 1. Dividing and radially enlarging xylem—a zone composed of (a) flattened, thin-walled cambium and neighboring xylem cells which are actively dividing but not enlarging in radial diameter, and (b) thin-walled cells enlarging in radial diameter but not yet showing lignification and thickening of the wall. 2. Maturing xylem—cells that have attained maximum radial enlargement but whose cytoplasm is still present and whose secondary walls are still thickening. 3. Mature xylem—cells that have completed development and are now a portion of the permanent wood layer. Mature xylem elements were further designated as earlywood or latewood tracheids according to Mork's classification (Smith and Wilsie 1961) or as flat latewood tracheids (Zahner et al. 1964).

The number of cells in each zone was counted in three continuous radial files in each wood sample. A seasonal trend of xylem development during 1965 was synthesized for individual trees by plotting the average count for each zone against the sample date and drawing a freehand curve through the points. Treatment trends were established by averaging the data of the three trees per plot. Because treatments were not replicated, inferences about interactions cannot be tested statistically. Main-effect differences in cell production by treatment and by bole position were tested by two-way variance analysis of plot averages and Tukey tests of simple contrasts.

RESULTS

Soil Water Regimes

Over the study period, the irrigated plot contained approximately three times as much water as the drought plot (17 to 27 cm more, fig. 1). The trenched control plot contained up to 12 cm more water than the drought plot, primarily in the surface 30 cm. Soil-water content in the drought-plus-irrigation plot was 2 to 8 cm more than in the continuous-drought plot through mid-July and about the same as that of the irrigated plot thereafter. Trees in the drought plot utilized water from below 122 cm, but not in sufficient quantities to maintain rapid cambial activity.

The desired soil-water conditions were maintained until September 10, when rains from a hurricane overflowed the catchment basin and accumulated in the open trench around the drought plot. The added water increased cambial activity. Cumulative diameter growth at breast height and at the base of the crown is shown in figure 2.

Amount of Cell Production

Drought appears to have reduced cell production at breast height. On drought plots the total number and radial width of tracheids during treatment in 1965 averaged 55 percent as great as before treatment in 1963-64 (tables 1 and 2); on other plots the number and width of tracheids was not altered much by treatment. Width of latewood cells appeared to be reduced by trenching. In trenched controls, cells averaged 73 percent as wide in 1965 as 1963-64, whereas they averaged 107 percent as wide in untrenched controls (table 2).

More cells, particularly earlywood cells (an average of 36 per radial file, as compared to 30), were produced at the base of the crown than at breast height (table 3). Tukey's method was used to test comparisons between main effects; treatments underscored by the same line did not differ

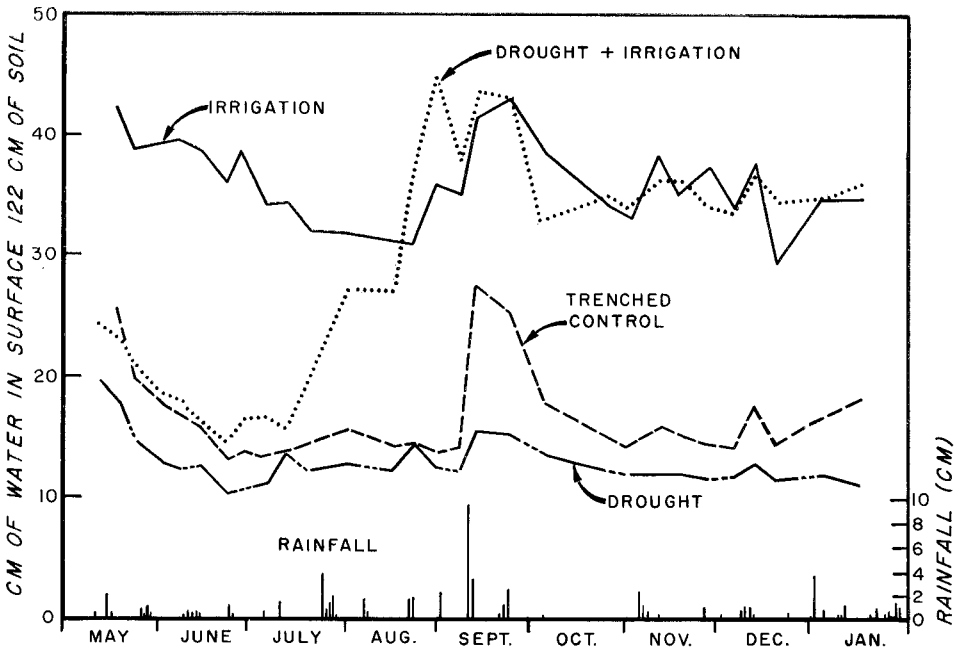

Figure 1.—*Soil-water content and daily rainfall, May 1965 to January 1966.*

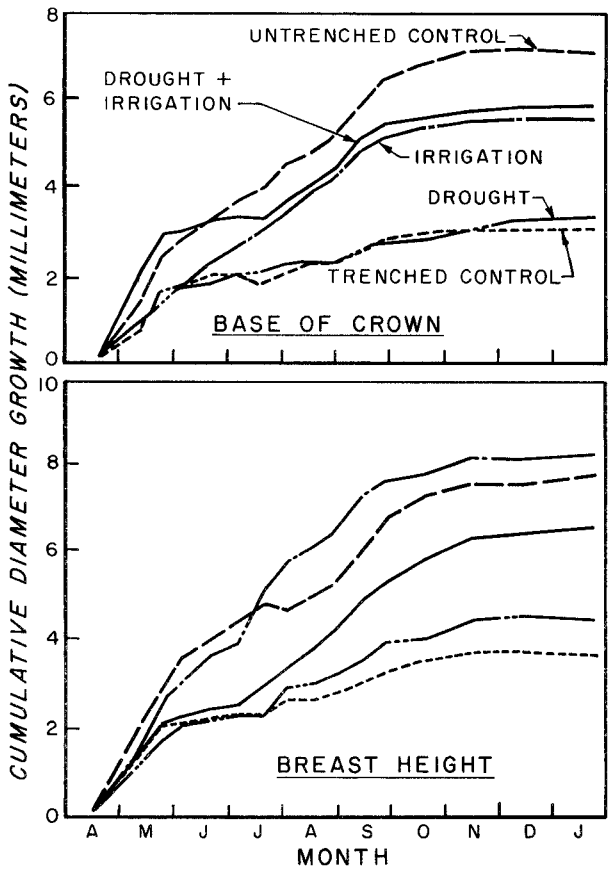

Figure 2.—*Cumulative diameter growth from April 1965 to January 1966.*

significantly (0.05 level):

Earlywood cell production					Total cell production				
UC	DI	I	D	TC	UC	I	DI	TC	D

All 12 trenched trees produced significantly fewer earlywood cells than the three untrenched controls, and trenched controls had significantly fewer earlywood cells than drought-irrigated trees. Latewood was too variable in our small sample to show significant differences even though drought trees developed less than half as many latewood cells at the crown base as at breast height (19 versus 43 cells).

Periodicity of Cell Production

Numbers of cells produced at breast height in 1965 are graphed in figure 3. In all trees cambial division had begun before the first wood samples were extracted on May 10 (23 to 31 cells had already formed) and continued into November. In drought trees, for example, 23 earlywood cells had been laid down by May 10, of which 6 were dividing and radially enlarging, 5 were maturing, and 12 were mature xylem. A similar pattern of cambial division was evident at the base of the crown (not illustrated). While the freehand curves do not reveal the variability of data, we think they illustrate

TABLE 1.—*Mean number and percentage of xylem elements for 1963-64 and 1965 annual wood layers at breast height*

Treatment	Earlywood			Latewood			Earlywood and latewood		
	1963-64	1965	1965 / 1963-64	1963-64	1965	1965 / 1963-64	1963-64	1965	1965 / 1963-64
	—No.—		Pct.	—No.—		Pct.	—No.—		Pct.
Drought	53	26	49	72	43	60	125	69	55
Irrigation	40	31	78	58	57	98	98	88	90
Drought + irrigation	33	29	88	53	54	102	86	83	96
Trenched control	19	24	126	43	37	86	62	61	98
Untrenched control	49	40	82	61	55	90	110	95	86

TABLE 2.—*Mean radial width of xylem elements for 1963-64 and 1965 annual wood layers at breast height*

Treatment	Earlywood			Latewood			Earlywood and latewood		
	1963-64	1965	1965 / 1963-64	1963-64	1965	1965 / 1963-64	1963-64	1965	1965 / 1963-64
	—Mm.—		Pct.	—Mm.—		Pct.	—Mm.—		Pct.
Drought	2.2	1.2	55	2.0	1.1	55	4.2	2.3	55
Irrigation	1.6	1.5	94	1.5	1.5	100	3.1	3.0	97
Drought + irrigation	1.5	1.4	93	1.3	1.5	115	2.8	2.9	104
Trenched control	1.0	1.1	110	1.1	.8	73	2.1	1.9	90
Untrenched control	2.3	2.1	91	1.5	1.6	107	3.8	3.7	98

TABLE 3.—*Tracheids per radial file, at breast height and base of live crown*

Treatment	Earlywood		Latewood		Total	
	At b.h.	At base of crown	At b.h.	At base of crown	At b.h.	At base of crown
	—Number—					
Drought	26	35	43	19	69	54
Irrigation	31	35	57	64	88	99
Drought + irrigation	29	39	54	50	83	89
Trenched control	24	28	37	44	61	72
Untrenched control	40	46	55	62	95	108

the general pattern of cell production as affected by treatments.

At breast height, Mork latewood cells first appeared in the maturing xylem between June 1 to 24, and flat latewood cells appeared as early as July 2 in trees subjected to drought:

Treatment	Initiation of cells	
	Mork latewood	Flat latewood
Drought	June 12	July 18, Nov. 4
Irrigation	June 14	Oct. 16
Drought + irrigation	June 5	July 2, Nov. 4
Trenched control	June 1	Nov. 4
Untrenched control	June 24	Nov. 11

Trenching seemed to have an effect, because latewood cells appeared 1 to 3 weeks sooner in all 12 trenched trees than in the three untrenched trees.

At breast height, Mork latewood cells first appeared in the permanent wood layer 2 to 3 weeks after becoming maturing xylem; at the base of the crown the transition occurred, on the average, 1 to 3 weeks later than at breast height. Date of latewood initiation varied widely among trees within plots.

Throughout the growing season the zone of dividing and radially enlarging xylem at breast height ranged from four to eight cells wide in all trees. In drought and drought-irrigated trees this zone was four to six cells wide, but after reduction of soil-water stress by irrigation and the hurricane it increased one to three cells in width, equaling the number of cells in the same zone of irrigated trees.

When soil-water stress was low, cell formation progressed at a uniform rate from May to October, and rates during summer often exceeded those in spring. Cell production diminished rapidly with increasing and prolonged soil-water stress. During July and August, for example, the rate at low stress was four times that at high stress, and the number of cells in the differentiating xylem of irrigated trees was 2½ times that of drought trees. When stress was changed quickly from high to low, the rate increased markedly but not until some 3 weeks later. In drought-irrigated trees, for example, the maturing xylem averaged three cells wide on July 26 (10 days after irrigation began) and 12 cells wide on August 16 (fig. 3). In drought trees, the maturing zone was four

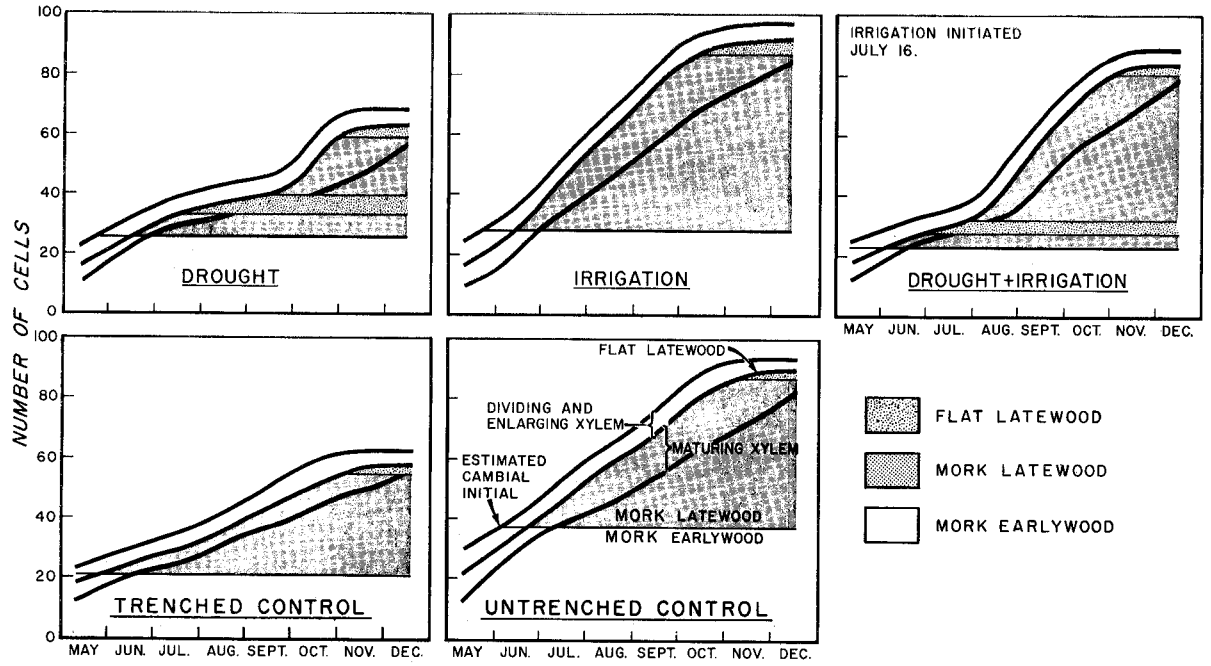

Figure 3.—*Curves of the seasonal development of the various zones of maturing tracheids in the xylem ring at breast height, in terms of the number of cells. The smoothed, freehand curves connect average number of cells for three trees, as determined by counts made on the 7th and 21st day of each month. As an example, the graph for untrenched control trees is interpreted as follows: Flat latewood cells (Zahner et al. 1964) first appeared in the wood sample extracted on November 11. Mork latewood cells first appeared in maturing xylem layer on June 23; these cells first appeared in the permanent wood layer on July 12, the date corresponding to the intersection of the earlywood-latewood zone boundary and the lower boundary of the maturing-xylem zone. This graphical technique, modeled after Whitmore and Zahner (1966), is valid only if all tracheids arise from the cambium initial (Bannan 1962, Wilson 1966).*

cells wide on September 27 (16 days after the hurricane) and 12 to 15 cells wide on October 18. In contrast, a similar spurt in cell production was not found at the base of the crown. In Monterey pines (*P. radiata* D. Don) similar in size to our loblolly pines, Shepherd (1964) detected resumption of rapid cell formation at breast height within a week after reduction of soil-water stress.

At breast height the number of cells in the zone of maturing xylem generally decreased from early May to late June, then increased until middle to late October. At its maximum, the zone consisted of approximately eight cells in trenched controls and 16 to 19 cells in all other trees. In irrigated and untrenched controls the zone began to expand earlier in June than in the other trees. At the base of the crown the zone contained a somewhat, but inconsistently, larger number of cells than at breast height.

Duration of Xylem Development

Time spent by cells in the differentiating xylem layer was estimated biweekly with Whitmore and Zahner's (1966) graphic-reconstruction technique. The uppermost curves in figure 3 represent the position of the estimated cambial initial plotted over time. Zones of dividing and enlarging xylem and of maturing xylem were scaled from this curve.

In drought trees, which produced proportionately fewer cells than other trees during 1965 as compared with 1963-64 (55 percent, table 1), the time spent by cells in the dividing and radially enlarging stage was greater than in other trees, ranging from 3 weeks under low soil-water stress in May and September to 8 weeks under high stress in July:

Treatment	Low stress	High stress
	— — *Weeks* — —	
Drought	3	8
Irrigation	4	2
Drought + irrigation	6	2
Trenched control	4	4
Untrenched control	3	3

In drought-irrigated trees, cells divided and enlarged for approximately 6 weeks during May and June

while enduring drought but for only 2 weeks in August and September while benefiting from irrigation. Cells of irrigated-only trees divided and enlarged for 4 weeks during May but for only 2 weeks thereafter. Untrenched controls exhibited a uniform pattern of cell division and enlargement, averaging approximately 3 weeks during the entire growing season. Trenched controls responded similarly, except that the period of division and enlargement averaged 4 weeks.

In all treatments, time spent by latewood cells in the maturing xylem increased gradually throughout the growing season, ranging from 2 weeks in early May to 10 weeks in late September (fig. 4):

Treatment	May	Sept.
	— *Weeks* —	
Drought and drought + irrigation	2.0	6
Irrigation	2.5	10
Trenched control	2.5	6
Untrenched control	2.5	8

In irrigated trees and untrenched controls, latewood cells remained in the maturing xylem approximately 3½ weeks longer than in trees subjected to trenching and drought. In all treatments new cells were evident in the maturing xylem on December 13 but not on January 24. Addition of new cells to the xylem by the cambium initial had ceased prior to November 15.

DISCUSSION AND CONCLUSIONS

Our finding that number and width of earlywood and latewood cells were reduced by drought agrees with the results of others (Foil 1961, Shepherd 1964, Smith and Wilsie 1961, Buijtenen 1958, Howe 1968). Drought affects cell production through its influence on tree-water stress, which reduces crown development. Larson (1964) has hypothesized that diminished crown activity restricts production of auxin, and thus reduces photosynthesis and impairs transport of carbohydrates to the cambial region.

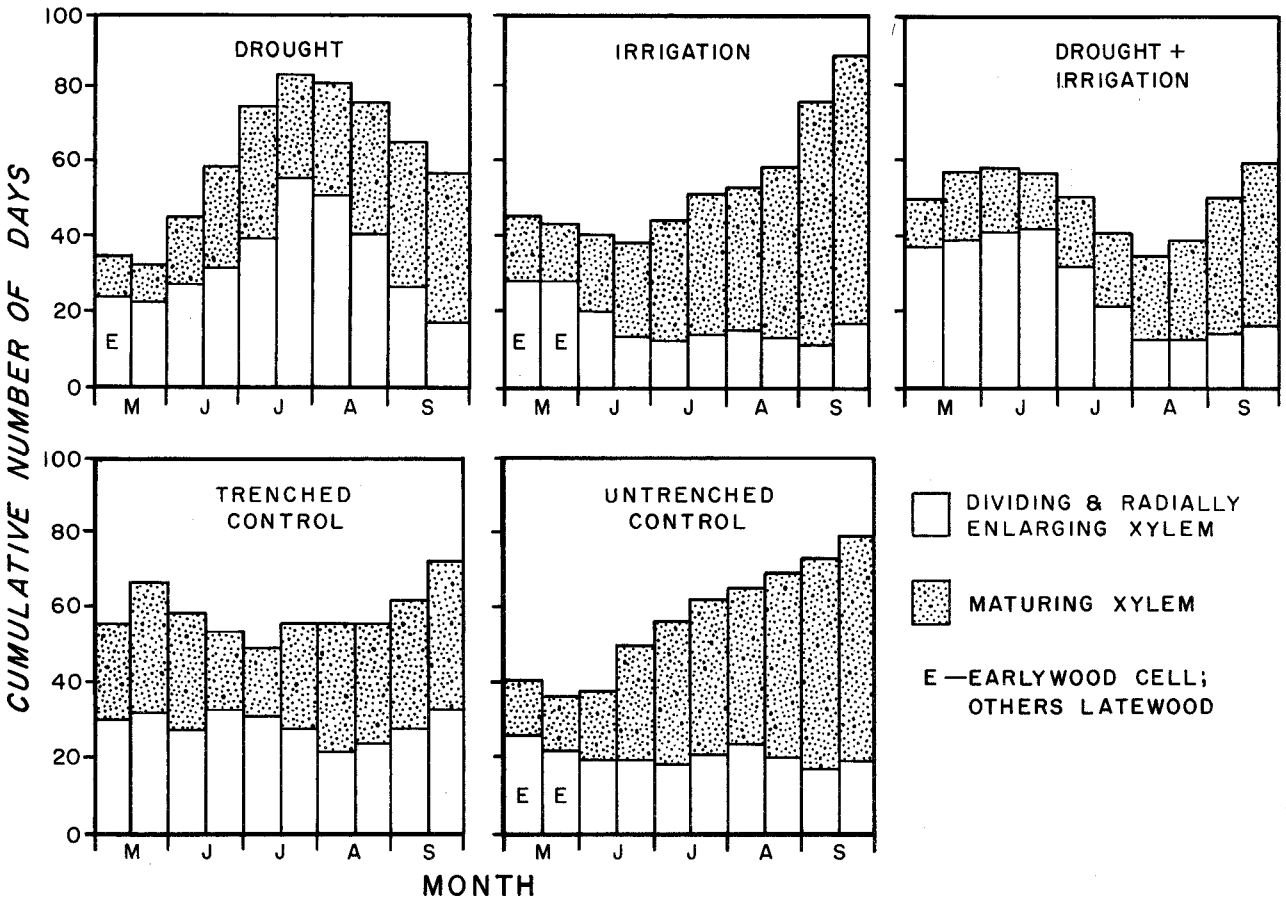

Figure 4.—*Time spent by cells originating after May 10 in the dividing and radially enlarging xylem and in the maturing xylem zones.*

Our results indirectly support this hypothesis.

Fewer earlywood cells and narrower latewood cells were produced in trenched than in untrenched controls. Trenching undoubtedly reduces the ability of trees to absorb moisture and, because some trees were closer to the edges of plots than others, the degree of root damage varied. Nevertheless, cell production in the trenched trees increased sharply after irrigation and the hurricane. The damage from trenching therefore was not so severe that it overcame the effects of moisture controls.

In general, the transition from earlywood to latewood tracheids was marked by a sharp increase in the time cells remained in the maturing xylem layer. Earlywood cells originating after May 10 (in drought, irrigated, and untrenched control trees) remained in this stage less than 2 weeks (fig. 4). Cells that qualified as latewood remained 2½ to 10 weeks, considerably longer than the 2½ to 4 weeks reported for red pine (Whitmore and Zahner 1966). Other research (Zahner et al. 1964) has shown that irrigation delays initiation of latewood. In our study, transition from earlywood to Mork latewood was little affected by irrigation; the transition occurred earlier in the 12 trenched trees than in the three untrenched trees presumably because trenching intensified internal water stress. In the six trees subjected to drought, flat latewood cells appeared in a narrow band in July, but subsequent relief of water stress stimulated production of wider latewood cells. Our results support those of other investigators who have reported that increases in soil-water availability accelerate cell production during latewood formation (Foil 1961, Howe 1968, Shepherd 1964).

In the six trees subjected to drought, cells enlarged less radially as soil-water stress increased, so that a narrow band of flat latewood cells formed prior to relief. In individual trees, these cells increased by as much as 50 percent in radial dimension within 3 weeks following resupply of water, but they were nevertheless classified as latewood by Mork's definition. Whitmore and Zahner (1966) emphasize that, independent of auxin, moisture stress affects cell expansion through photosynthesis and phloem transport; the mechanism is either a reduction in carbohydrate nutrition, reduction in growth substances transported from the crown, or direct physical retardation of cell division and enlargement. These authors believe that earlywood cells result when xylem derivatives become widely separated from the phloem and consequently are subjected to increasing competition for wall substrate. In our study, secondary cell wall synthesis was not greatly reduced even though cells that developed after the drought were rapidly separated from the phloem. The cells that increased their radial width after the drought were dividing and radially enlarging prior to reduction of soil-water stress. Thus they apparently retained the capacity to enlarge radially.

The length of time loblolly pine tracheids spent in the dividing and enlarging stage decreased as rate of cell division increased. Fewer and narrower tracheids were formed under drought than with ample moisture, and they took up to 6 weeks longer to enter the stage of maturing xylem. These observations agree with those of Whitmore and Zahner (1966) on red pine. High water stress may indirectly limit synthesis and transport of substances, thereby affecting cell wall plasticity, or it may directly reduce turgor, thus slowing enlargement. Cells initiated during stress appear to retain the capacity for expansion much longer than do nonstressed cells. Shepherd (1964) observed such a relation in Monterey pine.

Width of the zone of dividing and radially enlarging cells appears to be more uniform in loblolly than in red pine. Number of red pine cells decreased steadily during the growing season on both irrigated and drought plots (Whitmore and Zahner 1966, fig. 3). If auxins promote radial expansion of xylem cells, as some researchers believe, perhaps multiple flushing and consequent needle elongation maintain a constant amount of auxin in the cambium of loblolly pine. In red pine, shoot elongation is over by mid-June.

Width of the zone of maturing xylem, and time spent by cells in it, increased during the growing season — a pattern similar to that exhibited by red pine. In Monterey pine Skene (1969) has found that, late in the growing season, cell wall thickening lasts 8 to 10 weeks. In our study, time spent in this stage was inversely related to soil-water stress but lasted up to 10 weeks in contrast to a maximum of 4 weeks for red pine. Late in the growing season this zone narrowed more rapidly in red than in loblolly pine. Cells in the maturing xylem of loblolly pine retained cytoplasm until late December. The life of tracheid cells is apparently longer in loblolly pine than red pine.

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Induced drought decreased the total number of tracheid cells produced in a season and the radial width of latewood tracheids. Transition from earlywood to latewood occurred in early June and was little affected by irrigation.

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